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RESEARCH ARTICLE

Assessment of potential antagonists for anthracnose (*Colletotrichum gloeosporioides*) disease of mango (*Mangifera indica* L.) in North Western Ethiopia (Pawe)

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Mango (*Mangifera indica* L.) is considered as one of the most popular fruits among millions of people in the tropical area and increasingly in the developed countries. Anthracnose, caused by the fungus *Colletotrichum gloeosporioides*, is the most important pre- and post-harvest disease of mango. The objective of this research was to evaluate the prevalence of different promising antagonistic *Trichoderma* and *Bacillus* spp. on phyloplane of mango in Ethiopia and to evaluate their antagonistic potential against the pathogen. A total of 19 mango fields were surveyed and anthracnose affected all fields. Culture studies on potato dextrose agar for evaluation of antibiosis activity of *Trichoderma* spp. and *Bacillus* spp. revealed that they have inhibitory and lytic effect on *C. gloeosporioides*, which is an indication of their potential biocontrol agent for management of mango anthracnose as an alternative to chemical control. Significant differences ($p < 0.05$) were observed among *Bacillus* isolates in causing lysis of pathogen mycelium, when inoculated on actively growing colony of *C. gloeosporioides*. Maximum reduction in growth rate of pathogen was observed with *Bacillus* spp. (B50), which restricted the growth to 2.7 mm compared to 8.3 mm in the control with 67.5% efficacies. There were similar effects ($p < 0.05$) among *Trichoderma* spp. in formation of inhibition zones and lysis by varying degrees up to 59.7% efficacies in reducing linear growth of the pathogen in dual culture.

Keywords: Anthracnose; *Colletotrichum gloeosporioides*; *Trichoderma*; *Bacillus*; *Mangifera indica* L.

1. Introduction

Mango (*Mangifera indica* L.) is an important fruit crop in tropical and subtropical countries of the world. Despite its wide cultivation, the average yield of Mango is quite low, because of many biotic and abiotic constraints. The low productivity of biotic constraints is mainly due to the associated disease problem. Akem (2006) showed that anthracnose is presently recognised as the most important field and post-harvest disease of mango worldwide. The ubiquitous fungus *Colletotrichum gloeosporioides* Penz. and Sacc. is the anamorphic asexual stage of the pathogenic fungus that causes *Colletotrichum* diseases as anthracnose.

Wang (2009) reported that besides having a large number of hosts, the importance of *C. gloeosporioides* Penz. and Sacc. fungi is also evident in the following three

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aspects: The distribution is worldwide irrespective of differences in climate, from temperate to tropical regions. Secondly, pathogens in this genus can infect both young plant tissues and ripe fruits, thus diseases can occur either in the field or in the storage room. The diseases of young leaves and stems reduce the photosynthesis and transportation of nutrients, thereby indirectly influencing the formation and quality of fruits. Once fruits (either young or ripe) are infected, direct economic losses will be encountered. Finally, Pathogens in this genus can infect the entire plant, including aboveground and underground parts.

Effective control of anthracnose diseases usually involves the use of one or a combination of the following practices: using resistant cultivars, cultural control, chemical control and biological control using antagonistic organisms (Nelson 2008). Anthracnose can be successfully controlled using pre- and post-harvest fungicide treatments, heat treatments, or combinations of fungicide and heat treatments (Jabbar et al. 2011).

Plant diseases are mostly controlled by the use of chemical pesticides and in some cases by cultural practices (Agrios 2005). However, the widespread use of chemicals in agriculture has been a subject of public concern and scrutiny due to the potential harmful effects on the environment, their undesirable effects on non-target organisms and possible carcinogenicity of some chemicals (Agrios 2005; Sun et al. 2011).The need for the development of non-chemical alternative methods to control plant diseases is also due to development of resistant races of pathogens, a gradual elimination and phasing-out of some available pesticides (Heydari & Pessarakli 2010).

Chemical control of anthracnose of mango is very expensive and a difficult task for the common farmers to determine the precise dose of the chemical for its application to the field. In addition to this, harmful effect of the fungicide is responsible for air, soil, water pollution and causes serious health hazards (Tasiwal 2008). Indiscriminate use of chemicals disrupts the natural ecological balance by killing the beneficial and antagonistic soil microbes (Jabbar et al. 2011). The general strategy of biological control is to use one living organism to control the diseases, the control agents may be antagonistic micro-organisms or even natural plant- and animal-derived compounds (Chanchaichaovivat et al. 2007).

Biological control of disease includes management of diseases by organisms including plants, but excluding man, using micro-organisms, changing growth conditions of the plants (cultural practices) and using resistant varieties (Evuen & Ogbebor 2008). It is a viable alternative to chemical control of phytopathogens, because chemical control method can decrease fruit quality due to their toxic substance residues that may remain present so much time in the fruit (Burt et al. 2009).

Biological control involves the use of micro-organisms (usually from the same habitat as the pathogen) that inhibit or prevent the development of the pathogenic micro-organism, due to their high specificity and environmental safety (Tongsri & Sangchote 2009). Among the biological antagonists, there are bacteria and yeasts, the latter generally tolerate many of the fungicides and chemicals used during pre- and/or post-harvest, which allows producers to use them in combination with low toxicity chemical fungicides (Govender & Korsten 2006). Concerning anthracnose in mango, Basha et al. (2010) reported the application of *Bacillus* spp. and *Pseudomonas fluorescens* for the control of latent infections and post-harvest mango anthracnose.

Yenjit et al. (2004) showed that antagonistic micro-organisms such as *Bacillus subtilis, B. cereus, B. licheniformis* and *P. fluorescens* can be used to control anthracnose disease. Evuen and Ogbebor (2008) also showed that *Trichocladium* spp. and *Trichophyton* spp. exhibited the highest antagonistic effects on *C. gloeosporioides.* The antagonist was

more effective especially in the control of post-harvest diseases when fruit was kept in cold storage to simulate export conditions (Govender & Korsten 2006).

Sharma et al. (2008) reported that several post-harvest diseases could be controlled by biocontrol methods such as microbial antagonists. Competition for nutrients and space is most widely accepted mechanism of their action. In addition, production of antibiotics, direct parasitism and possibly induced resistance in the harvested commodity are other modes of their actions by which they suppress the activity of post-harvest pathogens in fruits and vegetables (Haggag et al. 2011).

The objective of this study was to identify the potential antagonists to *C. gloeosporioides* from Ethiopia, which can be developed into commercial biocontrols for the integrated management of anthracnose of mango disease.

2. Materials and methods

2.1. Sample collection

Samples of mango plants, inflorescences, leaves and fruits, were collected in 2011 and 2012 cropping season from farmers' fields in six villages of Pawe district of Benishangul-Gumuz region, Ethiopia, located between 11019′59.47 N latitude and 36025′00.66 E longitude. Farms were selected randomly based on location, accesses to road and/or production importance.

2.2. Laboratory analysis and isolation procedures

Fungal pathogens are able to infect various plant parts such as roots, stems, leaves, flowers and fruits, inducing characteristic visible symptoms like spots, blights, anthracnose, wilts, rots, etc. Samples from infected parts were taken, and then washed thoroughly in sterile water; the causal fungi were isolated from plant tissues exhibiting clear symptoms. The infected tissues along with adjacent small unaffected tissue were cut into small pieces (2–5 mm squares) and by using flame-sterilised forceps, they were transferred to sterile Petri dishes containing tap water then to alcohol (90%) solution and then to distilled water for surface sterilisation of plant tissues at a period of 30–60 s. The pieces were aseptically transferred to Petri dishes containing standard medium, potato dextrose agar (PDA), at the rate of 3–5 pieces of tissues per Petri plate and incubated at room temperatures (25–27 °C) for pathogen development. In the infected stems, roots or fruits, the fungal pathogens may be present in the deep-seated tissues. In such cases, the infected tissues were washed with sterile water thoroughly, followed by swabbing with cotton wool dipped in ethanol (80%) and exposed to an alcohol flame (from spirit lamp) for a few seconds. Using flame-sterilised scalpel, the outer layers of tissues are removed rapidly and small pieces from the central core of tissues in the advancing margin of infection are cut. They were sterilised by dipping in alcohol (90%) and sterilised again by exposure to alcohol flame for a few seconds.

The Petri dishes containing appropriate nutrient medium, after transferring the sterilised infected tissues, were incubated at required temperature and for optimum period. Actively growing mycelium from the medium was transferred to agar slants for further studies as mentioned above. Antibiotics were incorporated in the media to prevent bacterial contaminants (Rivera-Vargas et al. 2006; Rondon and Albarracin 2006; Sangeetha and Rawal 2010). Various taxonomic keys, references and morphological examination of species were used to identify and compare all fungal isolates from infected tissue samples.

2.3. *Evaluation of bioagents for their biocontrol potential against* C. gloeosporioides

2.3.1. In vitro *screening*

Testing bacteria and filamentous fungal isolates were brought from the University of Gondar, Gondar town (GPC W No. 10441 IMI 395920 and IMI 395919) were replicated in suitable media and tested for antibiotic production and lytic activity against growth of *C. gloeosporioides*. Potential antagonists and *C. gloeosporioides* were inoculated in the same culture plate while control plates were inoculated with *C. gloeosporioides* alone. After incubation for 5 days at 25 °C, the colony diameter of *C. gloeosporioides* were measured as the average of two cross-diameters. The degree of inhibition of growth was calculated as a percentage of the colony diameter in the control plates. Plates were incubated further and observation was made for antagonists that could not yield inhibition zone, but which can check the growth of *C. gloeosporioides* colony upon contact and which can lyses back its hyphae.

2.3.2. *Testing antagonistic and lytic effects of potential bacteria*

2.3.2.1. Testing for antagonistic activity of Bacillus *spp. against* C. gloeosporioides. *Bacillus* spp. were tested for antibiotic production and inhibition effects on the test pathogen isolate by dual culture methods. Five millimetre diameter disc of five-day-old culture of the pathogen were placed near the periphery of Petri dish and a loopful of *Bacillus* spp. isolate was placed at the opposite periphery of the plate containing PDA (Islam et al. 2012; Kumar et al. 2012). The inoculated plates were incubated along with culture plates with no antagonistic isolate at 21 ± 1 °C for 5–10 days.

The experiment was replicated three times in completely randomised design. Data on growth inhibition zone and colony diameter of pathogen and *Bacillus* spp. were recorded for each plate and inhibition of mycelial growth of the pathogen over control without *Bacillus* was calculated.

2.3.2.2. Testing for lytic activity of Bacillus *spp.* Five millimetre discs of pathogen mycelium were placed on 15 ml of PDA in Petri plates and incubated at 21 ± 1 °C. After three days of mycelia growth, one loopful of the isolate of *Bacillus* spp. grown for 48–72 h was placed on the actively growing colony of the pathogen and incubated at 21 ± 1 °C for 15 days. Lysis of pathogen mycelium was examined periodically under a stereomicroscope (40× magnifications) and the width of lysed mycelia around the colony of the bacteria was measured. The experiment was conducted in completely randomised design and replicated three times. Culture plates having *C. gloeosporioides* but without *Bacillus* spp. were used as control.

2.3.3. *Testing effect of potential fungi species*

2.3.3.1. Antagonistic activity of Trichoderma *spp. against C. gloeosporioides.* The antagonistic activity of *Trichoderma* spp. from the University of Gondar (GPC W No. 10382 and 10383) was tested against *C. gloeosporioides*. The *Trichoderma* spp. were tested for antibiosis activity to *C. gloeosporioides* on PDA in 9 cm Petri dishes. Three Petri dishes were inoculated with 2 mm mycelial disc from the edges of an actively growing colony of *C. gloeosporioides* on one side, with a similar sized disc of fungal antagonists on the other side, and incubated at 21 ± 1 °C. After 72 h of growth, the inhibition zones at the junction of colonies of fungal antagonist and pathogen were measured. In order to test for lysis of *C. gloeosporioides* colony, molten PDA medium

cooled to about 45 °C was poured into sterile culture plates and seeded with *C. gloeosporioides* propagule (104/ml conidia). After growth has occurred (medium covered by the mycelium), the selected antagonistic organism was spotted at three points over the test pathogen and incubated at 25 °C. After 10 days, lysis of *C. gloeosporioides* colony was examined periodically under stereomicroscope (50×) and the width of lysed mycelia around the colony of the lytic fungal isolate was measured. The experiments were replicated three times in completely randomised design. Culture plates with *C. gloeosporioides* alone were used as the control.

2.4. Data analysis

Data of biological control on inhibition zone (mm), colony growth diameter (mm), lysed diameter of pathogen mycelium (mm) and incubation period (days) were analysed using analysis of variance (ANOVA) to know the effect of fungal isolates on the growth of the pathogen and development of anthracnose. Least significant difference value was used to separate the treatment means. Data analysis was carried out using SAS, Ver. 9. Descriptive statistics were used during data analysis; ANOVA was performed and presented using tables.

3. Results

3.1. Morphological characterisation of C. gloeosporioides *isolates*

Morphological characterisation of isolates, colony appearance (texture, form and pigmentation), growth rate and conidial morphology was begun to be recorded after 48 h and then every 24 h, to distinguish *Colletotrichum* spp. that cause anthracnose of mango in Pawe district. On the day that the fungal colony fully covered the plate, the colour of each isolate colony was described using Rayner's (1970) mycological colour chart. Texture of aerial mycelium, the nature of colony edges and zonation were also described. Colony texture was recorded as either appressed with sparse aerial mycelium, flocculose with raised and slightly dense aerial mycelium, or floccose with raised and dense aerial mycelium. Colony colour was described as white, grey and pink. Colony shape was either uniform with smooth edges, irregular with rough edges or banded with sectors consisting of thin expansive mycelium. Isolates that grew at >9 mm/day were considered fast-growing, those that grew at between 7 and 8 mm/day were considered medium-growing, while those that grew at <8 mm/day were slow-growing. Significant differences were observed in the frequency of isolates with specific morphological or cultural traits in the villages (Table 1). In the case of colony colour, 49.2% of the isolates were white and 25.6% were grey while 16.6% were pink. Isolates with white and grey colour were identified as *C. gloeosporioides* while those with pink colour were *C. acutatum* (Peres et al. 2005). With respect to growth rate, 59.1% of isolates were medium-growing compared to 30.9% of slow-growing isolates, while 24.9 of isolates were fast-growing (Figure 1).

3.2. Tests for biological control

3.2.1. Antagonism test for Bacillus *against* C. gloeosporioides

Significant differences ($p < 0.05$) were observed among *Bacillus* spp. (Table 2) in formation of inhibition zones. The highest inhibition zone (4.4 mm) was observed from

Table 1. Number of *Colletotrichum* isolates from mango fields in Pawe district with different morphological characteristics. Six isolates were sampled at each of 19 fields.

Agro-ecological district and villages Pawe	No. of fields	Altitude range (m)	Colony texture ($p < 0.05$)			Colony color ($p < 0.05$)			Colony shape ($p < 0.05$)			Growth rate ($p < 0.05$)		
			Appressed	Floccules	Floccus	White	Grey	Pink	Uniform	Irregular	Sectoring	Slow	Medium	Fast
V4	3	1025–1039	11	4	3	10	8	0	8	10	0	7	11	0
V5	4	1040–1094	16	7	1	10	8	6	6	12	6	6	12	6
V6	3	1084–1087	12	4	2	12	6	0	10	6	2	3	10	8
V7	3	1079–1093	12	4	2	14	4	0	12	6	0	6	12	0
V12	3	1070–1074	10	5	3	12	6	0	16	2	0	8	10	0
V24	3	1142–1205	12	6	0	13	5	0	10	6	2	5	12	1
			$X^2 = 29.8$, d.f. = 2, $p < 0.05$			$X^2 = 28.7$, d.f. = 2, $p < 0.05$			$X^2 = 19.8$, d.f. = 2, $p < 0.05$			$X^2 = 20.5$, d.f. = 2, $p < 0.05$		

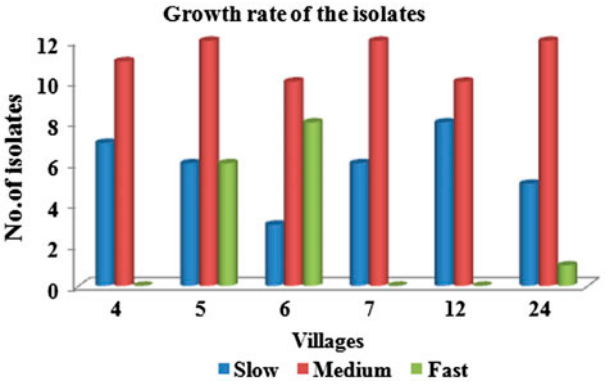

Figure 1. *In vitro* growth rate of *Colletotrichum* isolates collected from Pawe district.

Table 2. Performance of *Bacillus* spp. inhibition and lysis effect against *C. gloeosporioides* on PDA.

Bacillus codes	*Bacillus* colony reaction			*Bacillus* colony growth of 96 h (in mm)	Mean radial growth of *C. gloeosporioides* at 96 h (in mm)	
	Inhibition (%)	Lyses	Efficacy (%)			Control
B41	3.1^{b}	2.4^{b}	55	2.4^{a}	5.1^{d}	7.1
B42	3.4^{b}	2.6^{b}	66.6	2.5^{a}	5.3^{d}	7.5
B43	2.5^{b}	2.8^{b}	50	4.1^{c}	3.7^{b}	8.1
B50	3.1^{b}	4.1^{d}	67.5	2.6^{a}	2.7^{a}	8.3
B51	2.6^{a}	3.1^{c}	55	2.7^{a}	3.6^{b}	6.3
B52	3.4^{b}	2.5^{b}	57.5	2.8^{ab}	4.5^{c}	6.5
B53	3.1^{b}	3.2^{c}	60.5	3.3^{b}	3.7^{b}	7.5
B54	4.1^{c}	2.7^{b}	45.9	4.1^{c}	2.7^{a}	7.5
B61	4.4^{c}	3.3^{c}	30	4.1^{c}	3.8^{b}	6.3
B62	4.3^{c}	1.6^{a}	50	3.1^{b}	3.4^{b}	6.3
B63	4.1^{c}	2.3^{b}	45.3	3.5^{b}	2.6^{a}	6.5
B71	3.6^{b}	2.6^{b}	54.2	3.2^{b}	3.8^{cb}	7.2
B72	4.1^{c}	2.7^{b}	58.8	2.7^{b}	3.2^{b}	6.5
B73	3.7^{b}	3.1^{c}	60.5	3.1^{b}	4.6^{c}	7.5
B83	3.7^{b}	2.8^{b}	59.4	3.3^{b}	3.8^{cb}	7.9
Mean	3.5^{b}	2.8^{b}		3.1^{b}	3.8^{cb}	7.1
LCD (5%)	1	1		1	1	

Notes: Efficacy was calculated according to the following formula: $[C - B/C] \times 100$, where C is linear growth of the control and B is linear growth of the *Bacillus* isolate (Asran-Amal et al. 2005). Similar superscript letters indicates no significance levels, Difference in superscript letters indicates significance variation levels.

isolates B61 (4.4 mm) and B62 (4.3 mm), respectively. Most of *Bacillus* spp. produced 3 mm or higher inhibition zones. The lowest inhibition zone (2.5 mm) was observed with isolates B43. All tested isolates produced inhibition zones and reduced growth of in *C. gloeosporioides* the culture. Maximum reduction in growth rate of pathogen was observed with B50, which restricted the growth to 2.7 mm compared to 8.3 mm in the control with 67.5% efficacies. Three isolates restricted growth to less than 3 mm and showed 45.3–67.5% efficacy. Significant differences ($p < 0.05$) were observed among *Bacillus* spp. in causing lysis of pathogen mycelium, when inoculated on actively

growing colony of *C. gloeosporioides*. The lysed areas formed by antagonistic isolates ranged from 1.6 to 4.1 mm. The highest and the lowest lysed areas were observed with *Bacillus* isolates B50 (4.1 mm) and B62 (1.6 mm each), respectively.

Efficacy was calculated according to the following formula: $[C - T/C] \times 100$, where C is linear growth of the control and T is linear growth of the *Trichoderma* isolate (Asran-Amal et al. 2005).

3.2.2. In vitro *antagonism test for* Trichoderma *against* C. gloeosporioides

Culture studies on PDA for evaluation of antibiosis activity of *Trichoderma* spp. revealed that all inhibited growth of *C. gloeosporioides* and exhibited inhibition zone at the junction with the pathogen.

Highly significant effects ($p < 0.05$) were observed among *Trichoderma* spp. (Table 3) in formation of inhibition zones (Table 3). Out of 15 *Trichoderma* spp. tested against *C. gloeosporioides*, 10 of them produced 3 mm or higher inhibition zone. *Trichoderma* spp. reduced the growth of *C. gloeosporioides* colony by varying degrees. For example, isolates T411, T431 and T543 of *Trichoderma* were significantly more efficient in suppressing linear growth of *C. gloeosporioides*; however, T411 was more efficient (59.7%) among others.

3.2.3. *Lytic potential* Trichoderma *against* C. gloeosporioides

All the *Trichoderma* spp. when placed on mycelium of *C. gloeosporioides* caused lysis to varying extent. Isolates T431, T532, T543, T712 and T733 caused more than 2.5 mm

Table 3. Performances of *Trichoderma* spp. inhibition and lysis effect against *C. gloeosporioides* on PDA.

Trichoderma codes	*Trichoderma* colony reaction		*Trichoderma* colony growth of 96 h (in mm)	Mean radial growth of *C. gloeosporioides* at 96 h (in mm)	
	Lyses	Efficacy (%)			Control
T411	2.4^{ab}	59.7	3.4^{a}	4.5^{cd}	8.4
T431	2.7^{b}	47.3	4.1^{ab}	3.6^{b}	7.5
T511	2.4^{ab}	43	4.3^{ab}	3.1^{ab}	7.6
T521	2.2^{a}	30	4.6^{b}	4.1^{c}	6.8
T522	2.4^{ab}	32.9	5.6^{c}	2.5^{a}	8.5
T532	2.7^{b}	34.2	5.1^{bc}	3.2^{ab}	7.5
T543	3.1^{c}	47.1	4.5^{b}	2.5^{a}	8.6
T611	2.1^{a}	51	3.9^{ab}	3.5^{b}	8.2
T621	2.4^{ab}	42.5	5.1^{bc}	4.2^{c}	8.6
T632	2.3^{a}	33.3	5.2^{bc}	2.6^{a}	7.7
T712	3.2^{c}	38.4	4.8^{b}	3.6^{b}	7.7
T722	2.5^{ab}	26.6	5.3^{bc}	3.2^{ab}	7.6
T731	2.1^{a}	33.3	5.3^{bc}	3.5^{b}	7.7
T832	2.5^{ab}	31.7	5.6^{c}	4.1^{c}	8.3
T733	2.6^{b}	24.3	5.5^{c}	3.6^{b}	7.4
Mean	2.5^{ab}		4.9^{b}	3.4^{b}	7.8
LCD (5%)	1		1	1	

Notes: Efficacy was calculated according to the following formula: $[C - T/C] \times 100$, where C is linear growth of the control and T is linear growth of the *Trichoderma* isolate (Asran-Amal et al. 2005). Similar superscript letters indicates no significance levels, Difference in superscript letters indicates significance variation levels.

of lysis around them. Significant differences ($p < 0.05$) were observed among *Trichoderma* isolates in causing lysis of pathogen mycelium, when inoculated on actively growing colony of *C. gloeosporioides*.

The lysed areas formed by the different antagonistic isolates ranged from 2.1 to 3.2 mm. The highest and the lowest lysed areas observed with isolates T712 and T731 were 3.2 mm and 2.1 mm, respectively.

4. Discussion

There were significant differences in the frequency of isolates with specific morphological or cultural traits in the villages (Table 1). The key morphological features of the genus *Colletotrichum* are acervular conidiomata, often with setae (dark-pigmented, unbranched, thick-walled sterile hyphae usually pointed at the tip), elongated slimy conidia and the presence of appressoria which are thick-walled swellings at the end of a hypha or germ tube, useful for attaching the fungus to host surface before penetration of the tissue (Cano et al. 2004).

Morphological traits are highly variable among isolates; the morphology of *Colletotrichum* colonies varies within and among groups, depending on culture medium, substrate and environmental conditions, which affect stability of morphological traits. Regarding colony colours, 49.2% of the isolates were white and 25.6% were grey while 16.6% were pink. Isolates with white and grey colour were *C. gloeosporioides* while those with pink colour were *C. acutatum* (Peres et al. 2005). *Colletotrichum acutatum* has been reported as heterogeneous species (Wharton & Diéguez-Uribeondo 2004), which was difficult to distinguish morphologically from *C. gloeosporioides* as both exhibit extensive cultural variability and have overlapping host ranges. Thus, it would appear that *C. acutatum* has a broad host range, at least among fruit crops, and relatively non-host specific. With respect to growth rate, 59.1% of isolates were medium-growing compared to 30.9% of slow-growing isolates, while 24.9% of isolates were fast-growing.

An alternative approach to the use of fungicides for controlling plant pathogens is to replace them, at least in part, with antagonistic micro-organisms (Asran-Amal et al. 2005). The results of antagonism test for *Bacillus* against *C. gloeosporioides* showed the biocontrol potential of *Bacillus* spp. The evidences for this were the inhibition and lysis observed against *C. gloeosporioides* with maximum efficacy of 67.5% in reduction of linear growth of anthracnose isolates on dual culture (Table 2).

The entire *Bacillus* spp. used in this study as antagonists were significantly reduced the linear growth of anthracnose isolates on dual culture ($p < 0.05$). This suggested that most bacteria on leaf surface, fruit skin and blossom of mango were the potent antagonist of *C. gloeosporioides*. Similar evidence was reported by (Yenjit et al. 2004). The formation of clear zones on an agar plate revealed that bacterial antagonists (*Bacillus* spp.) could produce antibiotics with lytic potential against mycelial growth of *C. gloeosporioides*. This indicated the important role of bacterial metabolites in the inhibition of mycelial growth of *C. gloeosporioides*. Sahile et al. (2009) reported that *Bacillus* spp. were promising bacterial antagonists for controlling plant diseases, due to their simple nutritional requirements, ability to colonies dry surfaces for long period of time, rapid utilisation of many of the available nutrients and resistant endospores to withstand many of the environmental hazards.

In vitro antagonism test for *Trichoderma* against *C. gloeosporioides* also showed significant difference in reducing the linear growth of anthracnose isolates on dual culture ($p < 0.05$) through inhibition and lysis, which is an indication for antagonistic

potential of the fungi. This shows that all of *Trichoderma* spp. used for the study shown varying levels of antagonism against *C. gloeosporioides* on dual culture. The dual culturing of pathogen with *Trichoderma* revealed clearly potential of control in the isolates. Most isolates of *Trichoderma* produced 2.1 mm or higher inhibition zone on agar medium. These might be due to production of antibiotics or extracellular enzymes, which inhibited growth of the pathogen. In the activity of biological control, micro-organisms' action is not limited to direct influence on the target diseases, in addition to direct effect, they also enhance the resistance of the plants.

A report by Sahile et al. (2009) showed that *Trichoderma* strains are known to control pathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis, or directly by mechanisms such as mycoparasitism. Heydari and Pessarakli (2010) also suggested that *Trichoderma* spp. exhibit predatory behaviour under nutrient-limited conditions producing a range of enzymes that are directed against cell walls of pathogenic fungi, while chemical produced by the fungus such as xylanase is used for induction of host resistance. Similar results by Harman et al. (2004) and Kumar et al. (2012) also suggested that *Trichoderma* spp. inhibit the growth of fungal pathogen by producing sensing enzymes, antibiotics that release cell-wall fragments from the hyphae of the target pathogen and the production of a number of synergistic cell-wall-degrading enzymes and other substances that exhibit a wide antimicrobial spectrum.

5. Conclusion

Despite the pretence of limitation to the study, especially absence of molecular tools for determination of genetic differentiation of *Colletotrichum* isolates, the following are recommendations forwarded based on the study findings. Test for biological activities of antagonistic bacteria (*Bacillus* spp.) and filamentous fungi (*Trichoderma* spp.) showed their inhibitory and lytic effect on *C. gloeosporioides*, which is an indication for their biocontrol potential against mango anthracnose as an alternative to chemical control.

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